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Acute effects of light on the brain and behavior of diurnal *Arvicanthis niloticus* and nocturnal *Mus Musculus*

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Abstract

Photic cues influence daily patterns of activity via two complementary mechanisms: (1) entraining the internal circadian clock and (2) directly increasing or decreasing activity, a phenomenon referred to as “masking”. The direction of this masking response is dependent on the temporal niche an organism occupies, as nocturnal animals often decrease activity when exposed to light, while the opposite response is more likely to be seen in diurnal animals. Little is known about the neural mechanisms underlying these differences. Here, we examined the masking effects of light on behavior and the activation of several brain regions by that light, in diurnal *Arvicanthis niloticus* (Nile grass rats) and nocturnal *Mus musculus* (mice). Each species displayed the expected behavioral response to a 1 h pulse of light presented 2 h after lights-off, with the diurnal grass rats and nocturnal mice increasing and decreasing their activity, respectively. In grass rats light induced an increase in cFOS in all retinorecipient areas examined, which included the suprachiasmatic nucleus (SCN), the ventral subparaventricular zone (vSPZ), intergeniculate leaflet (IGL), lateral habenula (LH), olivary pretectal nucleus (OPT) and the dorsal lateral geniculate (DLG). In mice, light led to an increase in cFOS in one of these regions (SCN), no change in others (vSPZ, IGL and LH) and a decrease in two (OPT and DLG). In addition, light increased cFOS expression in three arousal-related brain regions (the lateral hypothalamus, dorsal raphe, and locus coeruleus) and in one sleep-promoting region (the ventrolateral preoptic area) in grass rats. In mice, light had no effect on cFOS in these four regions. Taken together, these results highlight several brain regions whose responses to light suggest that they may play a role in masking, and that the possibility that they contribute to species-specific patterns of behavioral responses to light should be explored in future.

Keywords

masking; diurnality; nocturnality; cFOS; temporal niche

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1. Introduction

Light is a powerful environmental cue that can have a major impact on daily patterns of behavior and physiology by entraining the endogenous circadian pacemaker and through more acute mechanisms that lead to a process referred to sometimes as “masking” [1]. Disentangling the influences of these two processes can be difficult in natural conditions because in environments with rhythmic alteration of light and darkness masking and circadian mechanisms complement each other to coordinate an animal’s patterns of adaptation to the day/night cycle [2]. Early circadian biologists devised experimental protocols to measure the influences of these two systems on behavior, but in doing so they generally dismissed masking as an important biological process in its own right [3]. This is evident in the original definition of the term, “…certain (sometimes overlooked) experimental conditions can *obscure* the real Zeitgeber-mechanism. We may call them masking conditions” [4]. Masking, however, can reflect adaptive mechanisms that contribute to regulation of the daily patterning of activity, rather than processes that simply obscure the influences of the circadian system.

Masking is a complex process [e.g. 5–8] and is typically quite different in day- and night-active animals, as light is more likely to increase activity in the former (a process referred to as positive masking) and decrease it in the latter (a process referred to as negative masking [9]). The patterns of response to photic cues can also change across the day in different ways [e.g. 1,10]. Many experiments have documented the suppression of activity by light in nocturnal mice [11–13], rats [14], and hamsters [15]. In recent years, several studies have described masking in diurnal rodents, such as Nile grass rats [10, 16], degus [6–8, 17], Mongolian gerbils [18] and golden spiny mice [19]. In a recent study we directly compared the behavior of nocturnal mice and diurnal Nile grass rats exposed to the same light stimuli presented at the same times of day and found that the animals responded in opposite ways: the light that suppressed activity of mice increased it in grass rats [10].

Although several experimental approaches have been used to study the neural substrates of masking responses, relatively little is known about them. One approach has been to examine the effects of lesions of retinorecipient regions of the brain on acute behavioral responses to photic stimuli. The suprachiasmatic nucleus (SCN) has been implicated in masking through such studies, though its role has been somewhat controversial [5, 20–21] and effects that have been seen could be due to damage to cells in the surrounding region (the ventral subparaventricular zone, vSPZ) [21] or damage to retinal fibers that go through the region of the lesions but do not terminate in the SCN [22]. Other areas that have been implicated in masking through lesion studies include the intergeniculate leaflet (IGL) [23–24], the dorsal lateral geniculate nucleus (DLG) [25], visual cortex [26], and the olivary pretectal nucleus (OPN) [27–28]. Consideration of the effects of lesions of different retinorecipient areas of the brain led Redlin [2] to propose that multiple areas mediate masking of activity by light in nocturnal species.

A second approach to exploration of neural substrates of masking has focused on the recently discovered melanopsin-containing intrinsically photo-responsive retinal ganglion

cells (ipRGCs) and the brain regions to which they project. In mice, the masking response is absent when these cells are absent or reduced [29–31]. ipRGCs project to many areas of the brain [32], one or more of which is likely to be functionally linked to masking.

Finally, several studies of nocturnal rodents have used the immediate early gene cFOS to characterize responsiveness to light of cells in regions to which the ipRGCs project. Results from these studies have revealed considerable differences across regions, species, and strains, as summarized in Table 1. For example, in two strains of mice exposure to 1 h of light of the same intensity in the same lab elicited different cFOS responses within the IGL [36, 38]. There are very few studies that have examined light-induced cFOS activation in diurnal species and most of these have focused exclusively on the SCN [57–60]. Only two have looked outside the SCN in a diurnal species, and these have revealed light-induced increases in cFOS in the peri-SCN region of Nile grass rats [59] and the IGL of degus [58]. In diurnal animals, nothing is known about patterns of responsiveness to photic stimuli in other brain regions that receive input from ipRGCs.

In the current study we examined cFOS in several brain regions of animals exposed to light that triggered an increase in activity of grass rats and a decrease in mice. First, we looked at areas that receive direct input from the retina, including the SCN, vSPZ, IGL, lateral hypothalamus (LH), olivary pretectal area (OPT) and the DLG. These areas might produce acute effects on general activity via pathways extending to structures that regulate sleep/wake state [5], as light can rapidly trigger sleep in nocturnal mammals (e.g. mice [61–62]) and heighten arousal/alertness in diurnal ones (e.g. humans [63]). We therefore also examined responses to light in the ventrolateral preoptic area (VLPO), a sleep-promoting region that receives retinal input (32), as well as in three brain regions that stimulate arousal, the lateral hypothalamus (LH), dorsal raphe (DR), and locus coeruleus (LC). Although the latter areas receive little or no direct visual input they could respond to light via indirect projections from the retina.

2. Methods

2.1. Animals

Adult female grass rats (n=8) were obtained from the breeding colony at Michigan State University and adult male CD1 mice (n=10) were obtained from Charles River Laboratory (Wilmington, MA, USA). Female grass rats are anestrous in our standard laboratory conditions [64], and masking responses to light are the same in male and female grass rats [10]. All animals were maintained on a 12:12 light-dark (LD) cycle with 300 lux of white light during the light phase and <1 lux of red light during the dark phase. All animals were singly housed in Plexiglas cages (34×28×17 cm) equipped with an enrichment device (PVC, length: 8 cm, diameter: 6 cm); food (PMI Nutrition Prolab RMH 2000, Brentwood, MO) and water were available *ad libitum*. The Institutional Animal Care and Use Committee of Michigan State University approved all experimental procedures.

2.2. Experimental Procedures

All animals were kept on a 12:12 LD cycle unless otherwise noted; zeitgeber time (ZT) 0 refers to the time of lights-on. Activity levels were monitored via infrared motion detectors

(IRs, Visonic Tel Aviv, Israel), and all counts from them were recorded with the VitalView Program (Minimitter, Bend, OR, USA). After two weeks animals were assigned to either a control group (grass rat: n=4, mice: n=5) or a group that was exposed to a one-hour light pulse (LP; grass rats: n=4, mice: n=5) beginning at ZT 14. All of these animals were perfused (see above) at the end of the light pulse (i.e. ZT 15).

2.3. Immunocytochemistry (ICC)

One series of sections from each animal (i.e. every third section) was processed with immunohistochemistry to visualize the distribution of cells containing the protein cFOS. The protocol for grass rats followed the same steps outlined for the CTβ reaction. In brief, sections were incubated in (i) 5% normal donkey serum (Jackson ImmunoResearch, West Grow, PA, USA), (ii) a primary rabbit anti-cFOS antibody (1:50,000, Santa Cruz Biochemistry, Santa Cruz, CA, USA), (iii) biotinylated donkey anti-rabbit antibody (1:200, Jackson ImmunoResearch) and (iv) the ABC complex (Vector Laboratory). From this point the procedure differs from that used for the CTβ. Sections were rinsed in a 0.14M acetate buffer (pH 7.2), and then reacted in a mixture of DAB (0.5mg/mL) and nickel sulfate in 0.14M acetate buffer (pH 7.2) with 3% hydrogen peroxide. Sections were then mounted, dehydrated, and coverslipped with dibutyl phthalate xylene (Sigma-Aldrich).

The procedure for processing tissue from the mice followed similar steps except that: (1) the tissue was rinsed in 0.1% PBT (PBS with .01% Triton X-100) rather than PBS, (2) the concentration of the primary antibody, rabbit anti-cFOS, was higher (1:20,000), and (3) the tissue was incubated overnight in the ABC solution. Finally, the DAB reaction was carried out in a Trizma buffer. A second series of brain sections from each animal was stained with cresyl violet and used to delineate regions of interest for analysis.

2.4. Data Analysis

To analyze the behavioral data, Vital View files were converted into actograms via ClockLab (Actimetrics, Wilmette, IL, USA) and raw data were transferred into Microsoft Excel. The actograms provided visual confirmation of masking behavior during the light pulse, while Excel allowed for quantitative assessment of the data. To statistically compare activity between groups, the raw data were first converted into percentages (activity during the hour before sacrifice/24 hr of baseline activity) and then arcsine transformed. For each species the values from light pulsed and control animals were compared with independent-sample t-tests using SPSS.

Counts were conducted by two observers, one for the grass rat study and the other for the mouse study. Observers were blind to the experimental condition of the animals; selected sections were counted by both observers to ensure that the same criteria were being used in identification of labeled cells. A light microscope equipped with a drawing tube was used to produce bilateral maps of cFos+ cells from at least one section through each area. Counts were done on two sections for the SCN, vSPZ, IGL, DLG, the ventrolateral preoptic nucleus (VLPO), the lateral hypothalamus (LH), locus coeruleus (LC) and DR. Counting boxes described in earlier reports were used to delineate regions sampled in the VLPO (190μm by 190μm; [65]), vSPZ (215μm by 160μm;[66]), LH (1200μm by 700μm; [67–68]), LC (400μm

by 700μm; [69]), and DR (150μm by 650μm; [69]). The remaining regions were outlined and all labeled cells within their borders were mapped and counted. The boundaries of the SCN and OPN were easily visualized, while the VMH, IGL and DLG were delineated with the aid of the nissl-stained tissue. Independent sample t-tests were used to determine if numbers of FOS+ cells differed between light-exposed and control groups; counts from each region, and from each species, were analyzed separately.

3. Results

3.1. Behavior

Exposure of grass rats to a 1 hr light pulse at ZT14 induced a marked increase in general activity. Specifically, among control animals, 4.69±0.67% of daily activity occurred during the hour beginning at ZT14, whereas among animals exposed to 1 hr of light at ZT14 this figure was significantly higher, at 15.72±4.59% ($t(5)=2.74$, $p=0.041$). The opposite response was observed in the mice. In this case, the percentages of daily activity occurring during the hour beginning at ZT14 were 17.66±3.52% among control mice but only 4.06±1.53% among light-exposed animals ($t(8)=3.75$, $p=0.006$). These results are consistent with previous findings [10].

3.2. cFOS expression in retinorecipent brain regions

Several different patterns of cFOS responses to light in retinorecipient regions were observed in the brains of mice and grass rats (Figure 1 & 2). One was evident in the SCN, where the number of cFOS+ cells increased in response to the light pulse in both species (grass rat: $t(5)=4.04$, $p=0.01$; mouse: $t(8)=4.77$, $p=0.001$). In the VMH, by contrast, neither species responded to the light; (grass rat: ($t(5)=1.10$, $p=0.32$; mouse: $t(8)=0.67$, $p=0.59$). In three other retinorecipient areas that we examined, the vSPZ, IGL and LHb, light induced an increase in cFOS in grass rats and had no significant effect in mice (grass rat: vSPZ $t(5)=7.57$, $p<0.01$, IGL $t(5)=31.22$, $p<0.001$ and LHb ($t(5)=5.67$, $p<0.01$; mouse: vSPZ: $t(8)=0.12$, $p=0.908$, IGL: $t(8)=0.22$, $p=0.828$ and LHb: $t(8)=1.33$, $p=0.220$). Numbers of cFOS+ cells within the ventrolateral geniculate nucleus of grass rats and mice showed the same pattern as in the IGL (data not shown). Finally, light induced an increase in cFOS in both the DLG and OPN of grass rats (DLG: $t(5)=3.61$, $p=0.012$; OPN: $t(5)=3.61$, $p=0.012$), and it led to a decrease in cFOS in these regions in mice (DGL: $t(8)=4.308$, $p=0.003$ and OPN: $t(8)=3.486$, $p=0.008$).

3.3. cFOS expression in arousal/sleep-related regions

We examined regions that are associated with sleep and arousal because light can directly inhibit sleep in diurnal species and induce it in nocturnal ones. In the grass rat, all arousal-inducing areas that we examined showed an increase in cFOS+ cells after the light pulse (Figure 3 & 4); this included the DR ($t(5)=7.67$, $p<0.01$) and the LC ($t(5)=7.11$, $p<0.01$). Among mice, there was no response to a light pulse in the DR ($t(8)=0.774$, $p=0.461$) or the LC ($t(8)=1.753$, $p=0.118$). In the LH (Figure 3 & 4), where orexin neurons are found, control grass rats had significantly lower numbers of cFOS+ cells than grass rats exposed to the light pulse ($t(5)=11.50$, $p<0.001$). Among mice, cFOS did not differ in the two groups ($t(8)=0.66$, $p=0.527$). Finally, in the VLPO, a sleep-promoting area of the hypothalamus,

light induced an increase in cFOS in the grass rats ($t(5)=5.87$, $p<0.01$) but had no effect in mice ($t(8)=0.74$, $p=0.481$).

4. Discussion

4.1. Technical issues

In the current study brain responses of mice and grass rats to light were similar in some regions but very different in others. Several technical issues need to be considered when interpreting these data. First, we did not do a dose response curve, as Dkhissi-Benyahya et al. (2000) [70] have done with gerbils. We therefore cannot say anything about the saturation points of the behavioral and brain responses that we saw, or whether they are different from those of gerbils. Furthermore, with more sensitive methods (e.g. [71]) we might have detected effects of light in some brain regions that did not exhibit a response in the current study. However, our most central question was how different regions of the brains of our diurnal and nocturnal species would respond to a stimulus that produces an increase in activity in the former and a decrease in the latter, which is something that our approach enabled us to do.

A second potential problem that must be considered is that the data from the grass rat study were obtained from females whereas for the mouse study we used males. The patterns we observed are not likely to be due to the sex difference, however, as the effects of 1 h light at ZT 14 on behavior are the same in male and female grass rats, and different in both compared to male mice ([10]; current results); we are unaware of any studies that have compared these patterns in males and females of the latter species. In fact, to our knowledge, there are no published data on whether sex influences masking or cFOS responses to light in any nocturnal rodent. Only three of the studies that we have been able to find on cFos responses to light (Table 1†) have used females, and in those three there was no assessment of whether the sexes differed. Mechanisms underlying the same behaviors in males and females could be somewhat different, and in the discussion below we do not assume that comparisons between the cFOS responses of our female grass rats and male mice reflect patterns that we would see from within sex/across species comparisons. However, it seems most likely that the key patterns are the same in males and females of the same species.

4.2. cFOS expression in retinorecipent brain regions

When considering the cFOS responses of different retinorecipient areas in grass rats and mice, four general patterns become apparent. (1) In the SCN grass rats and mice responded to light in the same manner. This result provides assurance that the fundamental parameters of the experimental manipulation (e.g. light intensities) and methods used to assess cFOS in this study were sound. (2) In the VMH there was no response in either species, confirming the fact that light-induced changes in cFOS that we observed did not reflect a simple widespread reaction to the stimulus, and can therefore provide insight into more focused functional responses to photic stimuli that induce masking. (3) In three retinorecipient areas, the vSPZ, IGL and LHb, light induced an increase in cFOS in grass rats but had no effect in mice. Thus, there is a relationship between a behavioral response to light and photic induction of cFOS in these regions in our grass rats, whereas the behavioral and cFOS

responses are dissociable in the mice, suggesting that masking of behavior by light does not depend on induction of cFOS in these areas in mice, though it might in grass rats. (4) Finally, in two brain regions, the DLG and OPN, the responses seen in mice and grass rats were in opposite directions. This raises the question of whether such cells might contribute to differences in functional responses to light in these two species.

The implications of these patterns are discussed in greater depth below, but first it should be noted that in some regions the cFOS responses observed in our study were different from those described in some earlier published reports, and that results among those studies are not always consistent. Table 1 summarizes key elements of experimental design and the results that are described in the research literature on cFOS responses to photic stimuli in other rodents. Many potentially important experimental parameters vary among these studies, including light intensity, time of day at which animals were exposed to light, the duration of the light pulse and the strain of mouse used in these studies.

4.2.1. SCN—In the SCN, we found that both species showed a clear increase in cFOS after the light pulse (Figure 1 & 2). This pattern of activation has been demonstrated before in both grass rats and mice pulsed with light during the dark phase of a 24 hr LD cycle as in the current study (Grass rat: [66,72]; Mouse: [42,73]), as well as when they are kept in DD and exposed to a pulse of light during the subjective night (Grass rats: [74]; Mouse: [27,75]). The focus of research on induction of cFOS in the SCN by light has been on its role in phase shifting of the endogenous circadian oscillator, and there is good evidence that it does play such a role in nocturnal rodents [77–78]. This is likely to be the case in grass rats as well, as effects of light on the circadian system in these animals are very similar to those seen in nocturnal species [78].

The possibility that light-induced cFOS in the SCN plays a role in masking has not been examined in any species, though the question of whether the SCN itself is important for masking has been addressed in numerous studies of SCN lesioned nocturnal rodents. One form of masking that is the same in nocturnal and diurnal species is the suppression of melatonin secretion by light at night, and this appears to depend on the SCN [63,79–80]. The role of the SCN in masking of behavior has been directly tested in only two studies of nocturnal animals (hamsters), and these produced conflicting results [20–21]. The presence of behavioral rhythms in SCN-lesioned animals maintained in a 24 hr LD cycle can also indicate the maintenance of masking, but here too, the results are mixed (Table 2); they are also complicated by the fact that SCN lesions may cause varying degrees of damage to the optic chiasm [96]. Although the nature of the role that the SCN might play in masking has not been definitively established in nocturnal species, Morin [5] makes a strong case that it is likely to be a central one. There are no data on these issues in diurnal species.

4.2.2. vSPZ—The vSPZ is one of the three retinorecipient regions in which light produced a robust increase in cFOS expression in grass rats but had no effect in mice. The induction of cFOS in this region by light has been seen previously in grass rats [74], as well as nocturnal rodents, including hamsters, rats, and mice [33–34,55,99]. Thus, the lack of a response in our mice is somewhat surprising. It is not likely to be due to technical difficulties, as in these same animals we were able to see clear behavioral responses to the

light, which also induced changes in cFos elsewhere in the brain. The differences among studies might be attributable to the fact that our strain of mice was not the same as those used in the two earlier studies [33–34]. In any case, the current results suggest that in our mice, the masking of activity by a photic stimulus can occur in the absence of a cFOS response of vSPZ neurons to that stimulus.

The question of whether the presence of a cFOS response of cells in the vSPZ reflects a role that this region may play in masking is more complex. It is an interesting area because it receives input from both the retina [32,100–102], and the SCN [103–105], and it projects to many of the same areas as the SCN [105–107]. It is thus well positioned to integrate direct photic signals from the retina with circadian signals, and to modulate the same functions that are regulated by the SCN. There is some evidence that the vSPZ may contribute to diurnality of the grass rat circadian system, as it exhibits rhythms in cFOS that are very different from those seen in nocturnal lab rats [108] and that persist in DD [66]. Lesions in this area also lead to a reduction in the ratio of activity during subjective day relative to night in these animals and to a decrease in the rate of reentrainment following a shift in the LD cycle [109]. The role of the vSPZ in masking, however, has not been directly tested in any species.

4.2.3. IGL—The second of the three retinorecipient regions in which light produced an increase in cFOS in grass rats but had no effect in mice is the IGL (Figure 1 & 2). Interestingly, cFOS is induced by light in the IGL of another diurnal species, the *Octodon degus*, [58]. There is good evidence that this is also the case in the hamster, but the data on rats and mice are inconsistent (Table 2) [36,38,43–47,48, 51,99,110]. This may be due to differences in the length of the light pulse to which animals were exposed, or to the strain of animal examined. Interestingly, Juhl et al. [50] found in rats that light triggered an increase in cFOS within the subset of IGL cells that contain enkephlin but not in neuropeptide Y-containing calls. One important question is whether light induces cFOS in the same subpopulations of IGL cells in diurnal grass rats as it does in nocturnal lab rats.

The patterns of responsiveness of grass rats and mice seen in the current study raise the question of whether the IGL might contribute to differences in their masking behavior. Several kinds of data suggest that this might be the case. As is the case with the SCN and vSPZ, the retinal input to the IGL comes from ipRGCs in mice [32], and these cells play an important role in masking [111]. Lesion studies have also produced interesting results relating the IGL to masking, in both nocturnal and diurnal animals. In nocturnal rodents, masking is retained under LD conditions after the IGL is lesioned [24,112–113] and in hamsters the masking response of wheel running to light is actually increased after the IGL has been destroyed [113]. The most striking effect of IGL lesions on masking, however, is that seen in diurnal grass rats, as Gall et al. [23] showed that destruction of the IGL completely reversed the direction of the masking response. That is, lesioned animals decreased their activity when presented with light, rather than increasing it. Results from lesion studies, as well as the cFOS response shown here, therefore suggest that the IGL plays an important role in the ability of grass rats to sustain a masking response typical of diurnal animals. It should be noted that the influence of light on cFOS within the IGL could be indirect, occurring, for example, via inputs that it receives from the from orexin cells in the lateral hypothalamus.

4.2.4. LHb—The LHb is the third retinorecipient brain region in which light increased cFOS in the grass rat but had no effect in mice. Only one other study that we are aware of has directly assessed effects of light on cFOS in this region in nocturnal mice, and that study found a large stimulatory effect [34]. On the other hand, in an LD cycle, cFOS in this region is higher during the night than during the day in nocturnal rodents, including mice, rats and hamsters [114]. It should also be noted that activity appears to induce a rise in cFOS in the habenula [114–116], and cFos in this region is associated with stress [117–118]. Perhaps differences in these parameters could account for the differences between our results with mice and those described by LeGates et al. [34].

The response of the habenula to light is of special interest because its roles in regulation of activity [115] and its projections to arousal-inducing areas of the brain [119–121] put it in a good position to mediate masking. *In vivo* electrophysiological studies in rats have shown that neurons in the habenula, particularly its lateral subregion, respond to acute presentation of light, and *in vitro* recordings reveal a circadian rhythm in its firing rate [122]. Though it is not yet clear whether the LHb contributes to masking, or to species differences in its manifestation, its responsiveness to light in grass rats (Figure 2) suggest that it is a region that should be explored further in efforts to better understand these issues.

4.2.5. DLG—The DLG is one of the two brain regions in which light induced an increase in cFOS in the grass rat and a decrease in mice (Figure 2). It should be noted that Prichard et al. (2002) saw no effect of a 2 hr pulse of light at midnight on cFOS in the DLG of rats. In contrast to these nocturnal rodents, grass rats exhibited a robust response to light, as has been reported in two other diurnal species, tree shrews [123] and Mongolian gerbils [124]. The DLG is an important part of the primary visual system [125–126], but it also plays a role in the masking behavior of mice, as lesions in this area enhance the inhibitory effect of light on activity in these animals [26]. The possibility that the DLG plays a role in masking in grass rats and other diurnal species has not yet been examined, but the data here suggest the possibility of interesting and important differences in the role that it might play in nocturnal and diurnal species.

4.2.6. OPN—The second region in which light increased cFOS in the grass rat and decreased it in mice was the OPN (Figure 2). In this case, the control mice appeared to have very high levels of cFOS, and light exposure diminished the number of cFOS+ cells by more than 50%. This result stands in contrast to that described in another nocturnal rodent by Prichard et al. [51], who reported that a 2 hr pulse of light at ZT19 induced a significant increase in cFOS within the OPN of lab rats; light during the inactive period had no effect in that study. The different responses of our mice and the rats of Prichard et al. [51] could be due to the times of day at which animals were sampled, or the OPN may not be the same with respect to the manner in which it responds to light in these two nocturnal species.

The focus of research on the OPN has been on its mediation of the pupillary light reflex [127] but the same characteristics that allow it to play that role are ones that could also enable it to contribute to masking. The OPN receives substantial input from melanopsin-containing cells in the retina [32], contains neurons that are capable of coding illumination levels [128] and it projects to at least one region that appears to play a role in masking, the

IGL [129]. Additionally, removal of the OPN interferes with masking of REM sleep by darkness in albino rats [27]. The role of this region in masking of general activity by photic stimuli has not been examined in either nocturnal or diurnal species.

4.3. cFOS expression in Arousal/Sleep-Related Regions

In all four arousal/sleep-related areas examined here light induced an increase in cFOS in grass rats but had no effect in mice. The implications of these species differences are discussed below, but it should be noted that in this case there are few published data with which to compare our results.

4.3.1. VLPO—In the *VLPO*, a region of the hypothalamus known to promote sleep in some species [130–131], we found that the same light that stimulated general activity actually increased cFOS in grass rats (Figure 3). The reasons for this paradoxical response are not clear, but one possibility involves the internal circuitry of the VLPO. The subset of cells in this region that actually stimulate sleep in nocturnal rodents are known to contain galanin, and cFOS is elevated in those cells during sleep in nocturnal rats [132]. Thus, it may be that in grass rats light activates a different subset of cells, perhaps even inhibitory interneurons that suppress galanin-containing cells in the VLPO, which could lead to a reduction in sleep.

In mice, we found that while light decreased activity it did not affect cFOS in the VLPO. Previous experiments examining light-induced cFOS in this area in mice have produced results that are somewhat contradictory. Lupi et al. [37], using RT-PCR, saw a significant light-induced increase in mRNA for cFos when mice were pulsed for 1 hr with light. However, Tsai et al. [40] saw no effect of light on overall levels of cFOS in this region but they did when they focused specifically on the sleep-promoting galanin-positive cells there. It should be noted that the VLPO is innervated by fibers originating in the retina in grass rats and mice [32,101], though there is evidence that the density of that retinal input is higher in nocturnal lab rats than grass rats (Nunez, unpublished results). Several investigators have suggested that this pathway may play a role in masking [2,37] but to our knowledge no one has directly examined this possibility.

4.3.2. LH, DR, LC—The LH, DR and LC are of interest in understanding the neural mechanisms of masking as each plays an important role in induction and maintenance of arousal [133–135]. In grass rats, all three of these brain regions responded to light, which may reflect their contribution to the light-induced increase in activity seen in these animals. In mice, however, there was a dissociation between the effects of light on activity and on cFOS in these regions. That is, the light that inhibited activity had no effect of cFOS in the LH, DR or LC. This result, which has been reported by others [39], may reflect a delay in the decay of cFOS relative to a decline in neuronal activity. Light-induced cFOS has been seen previously in the LH and DR of grass rats [136], as well as the DR of another diurnal murid rodent, the Mongolian gerbil [124]. Interestingly, the retina projects directly to the LH of grass rats [101], and to the DR of Mongolian gerbils [137]. It is tempting to speculate that these pathways play a role in the induction of cFOS and positive masking in these diurnal species.

5. Conclusions

The acute behavioral responses of nocturnal and diurnal species to light exposure, which may be modulated by time of day, are typically completely different, with the nocturnal animals decreasing activity and diurnal ones increasing it (e.g. [10]). Although little is known about neural mechanisms that are responsible for these differences, the present data highlight some brain regions that could play important roles. Here, we found that when grass rats and mice held under identical conditions were pulsed with light that had diametrically opposed effects on behavior, most retinorecipient and arousal-related brain regions responded differently. The only exception was the SCN, which likely reflects the fact that the relationships between photic stimuli, circadian rhythms and SCN function are very similar in nocturnal and diurnal species [78]. The differences in cFOS responses to light in other retinorecipient regions are more likely to reflect neural mechanisms that contribute to species differences in masking. Data on lesions of the IGL have provided support for the hypothesis that this region plays an important role in maintenance of positive masking of activity by light in these diurnal animals [23]. The present data raise the possibility that differential responsiveness of cells within the vSPZ, LHb, OPN and DLG may also contribute to the constellation of adaptive responses to light that distinguish diurnal from nocturnal species. Future experimental work will be required to assess this possibility, to establish the role of these brain regions in masking behaviors.

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Highlights

- Light induced changes in brains of diurnal grass rats and nocturnal mice.
- cFos responses of the two species differed in several retinorecipient regions.
- cFos was stimulated in sleep/arousal-related regions in grass rats but not mice.

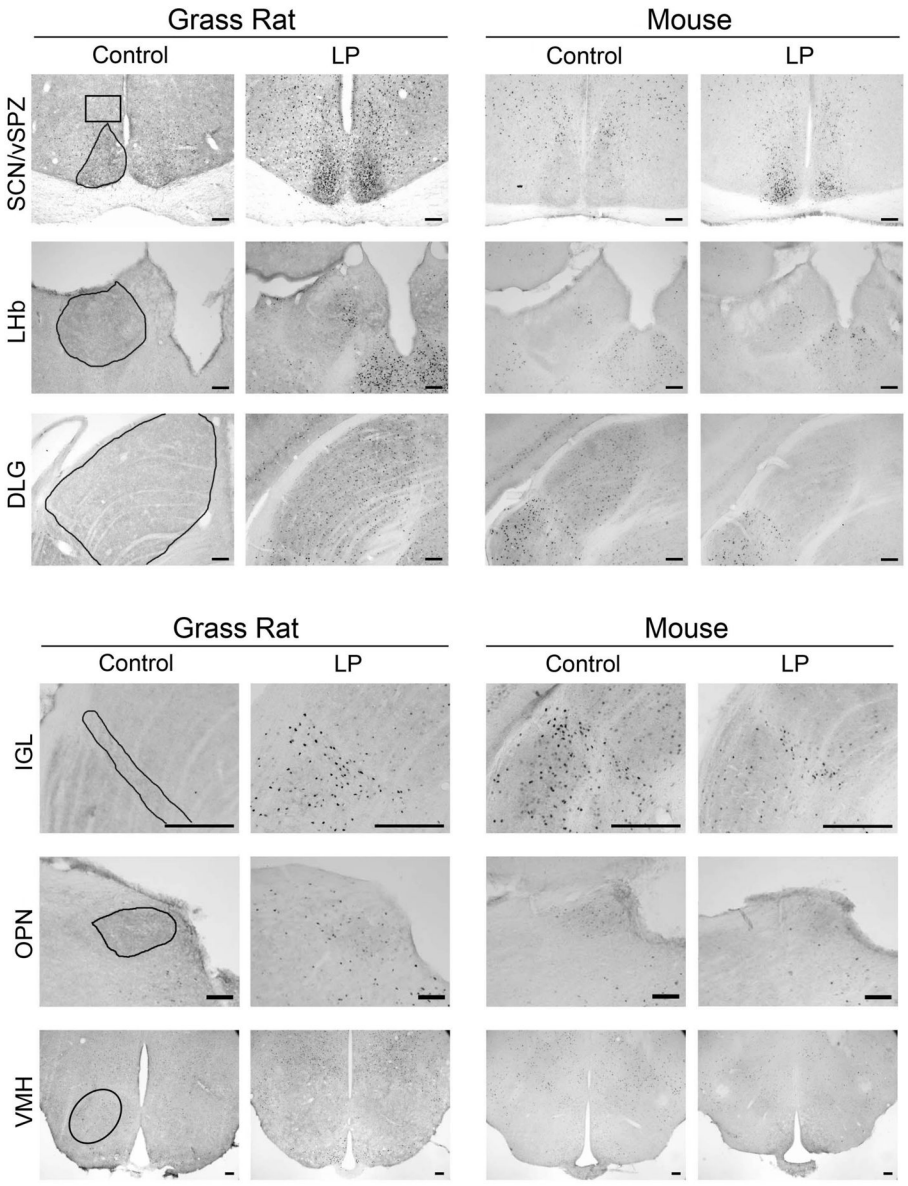

Figure 1.

Photomicrographs of cFOS in retinorecipient brain regions of grass rats and mice. In grass rats, on the left, cFOS was increased by the light pulse in all areas except the VMH, which remained unchanged. In the mice, on the right, cFOS was increased by the light pulse in the SCN, unchanged in the vSPZ, LHb, IGL, and VMH and decreased in the dLGN and OPN. Areas of interest are outlined in the first column; sampling areas are described in the text. Scale bar = 100μm.

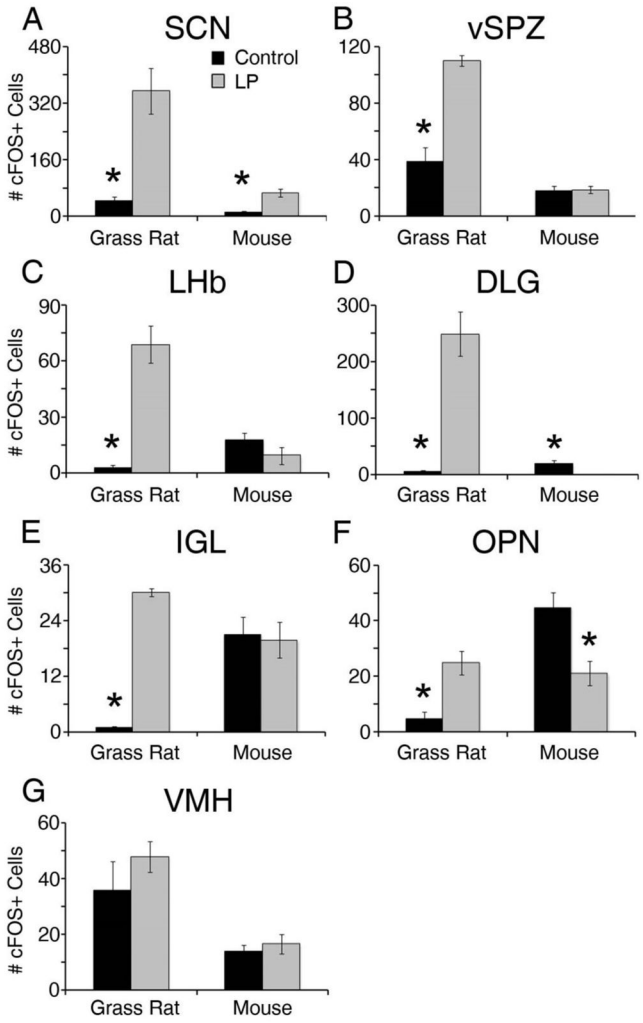

Figure 2.

Patterns in cFOS expression in retino-recipient brain regions of light-exposed and control grass rats and mice. Panel A shows a significant increase in activation within the SCN in both species. Three regions, the vSPZ (B), LHb (C) and IGL (E) responded with a significant increase in grass rats but not in the mice. Two regions, the DLG (D) and OPN (F) had opposite responses to light exposure in the two species, with mice experiencing a decrease and grass rats an increase in activation. Panel G shows no response within the VMH of either species. An asterisk (*) indicates significance $p < .05$.

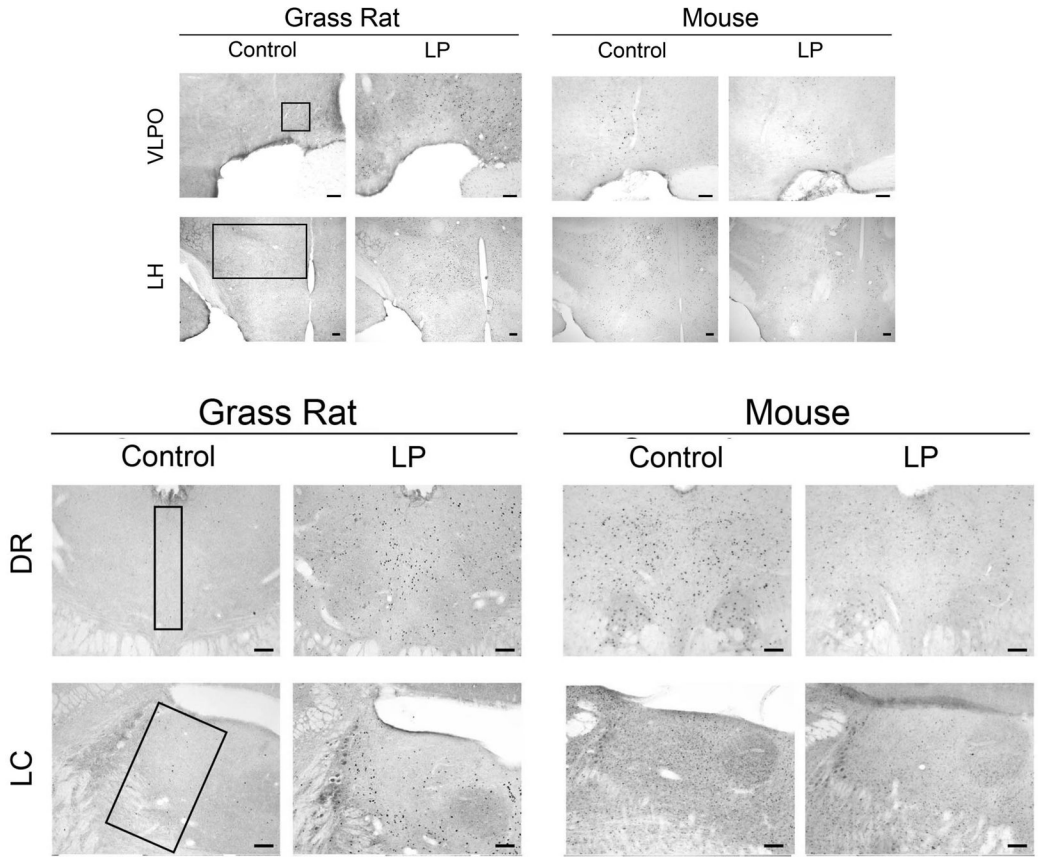

Figure 3.

Photomicrographs of cFOS in arousal/sleep-related regions. In grass rats, on the left, cFOS was increased by the light in all areas. In mice, on the right, light had no effect on cFOS in any of these areas. Regions of interest are outlined in the first column and sampling regions are described in the text. Scale bar = 100μm.

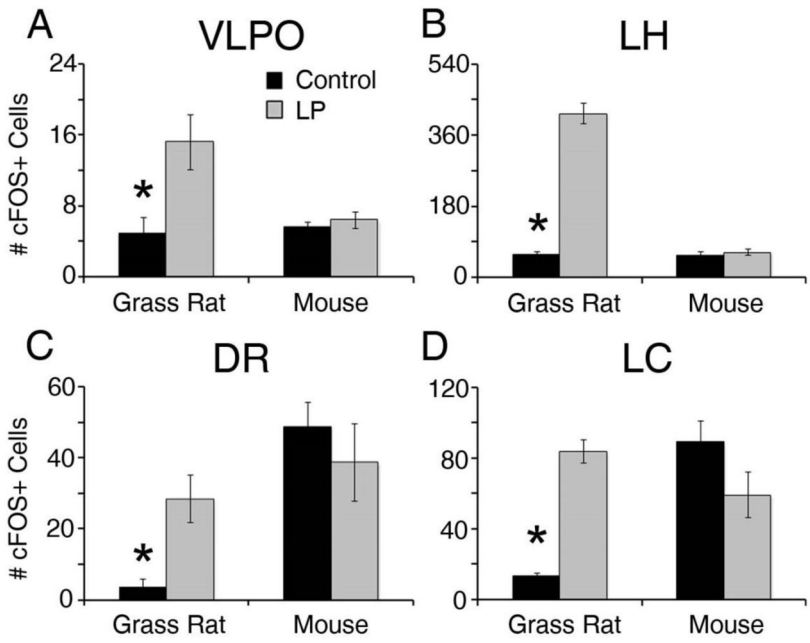

Figure 4.

Patterns in cFOS expression in arousal/sleep-related regions. In the grass rat, cFOS was induced by light in the VLPO (A), LH (B), DR (C), and LC (D). No response was observed in any of these arousal/sleep-related regions in the mouse. An asterisk (*) indicates significance $p < .05$.

TABLE 1

cFOS activation in response to light pulses in nocturnal mice, rats, and hamsters

Author	Strain	Time	Duration	Areas	Trend
(a) Mice					
Brooks et al., 2011 [33]	C57BL/6J	CT16	30min	SCN SPZ	↑ ↑
LeGate et al., 2012 [34]	B6/129 F1 hybrid	ZT14	10min	SCN SPZ LHb	↑ ↑ ↑
Lima et al., 2003 [35]	C57BL/6J & C3H/HeJ	Not Reported	1hr	OPN	↑
Lupi et al., 1999 [36]	C57BL/6J	CT16 LP and Perfused at CT17.5	15min	SCN IGL	↑ (=)
Lupi et al., 2008 [37]	Control: C3H Mutant: C57BL/6 & 129/SvJ hybrid	ZT16	1hr	SCN VLPO	↑ ↑
Lupi et al., 2012 [38]	C3H/He	ZT16	15min	SCN IGL	↑ ↑
Mendoza et al., 2010 [39]	C57BL/6J	CT12	30min	LH (ORX) Raphe (5-HT)	(=) N/A
Tsai et al., 2009 [40]	C57BL/6 & 129/SvJ hybrid	ZT15	1hr	SCN VLPO	(=) (=), ↑ GAL+ cell
Huerta et al., 1999 [41]†	C57BL/6J	CT16 &22	1hr	SCN	↑*
Masana et al., 1996 [42]	C3H/HeN	CT2,6,10,14,18, and 22	15min	SCN	↑
Delogu et al., 2012 [43]†	Not Reported	ZT19	60min	SCN	↑*
(b) Rats					
Rusak et al., 1990 [44]	Not Reported	D and L phases in DD, ZT4.5	30 or 60min	SCN IGL	↑* ↑*
Peters et al., 1996 [45]	Sprague-Dawley	Subjective Day & Subjective Night	2hr (FOS) & 30min (fos)	IGL	↑
Aronin et al., 1990 [46]	Sprague-Dawley	ZT4	4hr	SCN IGL	↑ ↑
Aronin et al., 1990 [46]	Sprague-Dawley	ZT4	4hr	SCN IGL	↑ ↑
Park et al., 1993 [47]†	Sprague-Dawley	CT16	1, 1.5, 2, 3hr	SCN IGL	(=) 30–90min, ↑ 2+
Janik et al., 1992 [48]	Sprague-Dawley	CT14	2hr	SCN IGL	↑*
Cha et al., 2011 [49]	Sprague-Dawley	Not Reported	1hr	DLG	↑*

Author	Strain	Time	Duration	Areas	Trend
Juhl et al., 2007 [50]	Wistar	ZT6, ZT14, ZT19	90min	IGL	↑ ZT6, (=) ZT14 and 19
Prichard et al., 2002 [51]	Fischer F344	ZT6 & 18 Control; ZT7 & 19 pulsed	60min	OPN IGL DLG	(=) ZT7, ↑ ZT19 ↑ ZT7 and (=) ZT19 (=)
(c) Hamsters					
Rusak et al., 1990 [44]	Not Reported	D and L phases in DD, ZT4.5	30 or 60min	SCN IGL	↑ night, (=) day ↑ night, (=) day
Janik et al., 1995 [52]	Golden	CT18	30min	SCN IGL	↑ ↑
Marchant et al., 1999 [53]	Golden	CT18.5	15 min	SCN IGL DR	↑ ↑ ↑
Muscat et al., 2006 [54]	Golden	CT19	5 min	SCN IGL	↑ ↑
Zhang et al., 1993 [55]	Golden	CT19	5 min	SCN IGL SPZ	↑* ↑* ↑*
Zhane et al., 1996 [56]	Golden	CT19	5 min	SCN	↑

*Indicates that the study did not quanitatively analyze the data

†Indicates that the study used both sexes

Table 2

Masking in 12:12 LD condition for animals with suprachiasmatic nucleus lesions

Author	Species	Strain/Species	Variable	Masking
Easton et al., 2004 [81]	Mice	C57B1/6J	GA, EEG, Temperature	N*
Tong et al., 2013 [82]	Mice	ddY	HR and Temperature	N
Stephan & Zucker, 1972 [83]	Rat	Sprague-Dawley	Drinking and Wheel running	N†
Coindet et al., 1975 [84]	Rat		EEG	N
Mistlberger et al., 1983 [85]	Rat	Sprague-Dawley	EEG	**Y**
Liu et al., 2012 [86]	Rat	Sprague-Dawley	EEG	N
Ibuka & Kawamura, 1975 [87]	Rat	Albino	EEG	N
Ibuka et al., 1977 [88]	Rat	Wistar	EEG	N
Aguilar-Roblero et al, 1986 [89]	Rat	Wistar	Drinking	N
Scheer et al., 2001 [14]	Rat	Wistar	Drinking, HR, GA	N*
Amir et al., 2004 [90]	Rat	Wistar	Wheel Running	N*
Zhang et al., 2004 [91]	Rat	Wistar	GA & Temperature	**Y**
Hu et al. 2007 [92]	Rat	Wistar	GA	N
Angeles-Castellanos et al, 2010 [93]	Rat	Wistar	GA & Temperature	N*
Warren et al., 1994 [94]	Rat	Long Evans	GA, HR, Temperature	N
Wachulec et al, 1997 [95]	Rat	Long Evans	Drinking, Temperature, GA	**3Y 5N**†
Mistlberger et al., 1992 [96]	Hamster	Golden Syrian	Wheel running	**10Y 2N**†
DeCoursey et al. 1997 [97]	Ground Squirrels		Wheel running	**Y**
Fuller et al, 1981 [98]	Squirrel Monkey		Drinking and Temperature	**Y**
Sato & Kawamura, 1984 [98]	Chipmunk	Siberian	Wheel running	**1Y 3N**

Y Animals maintain masking response to light after lesion

N Animals no longer mask to light after lesion

† Extensive damage to the Optic Chiasm

* Used lack if rhythm as criteria for good lesion